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CLINICAL INVESTIGATION **Breast**

THE RISK OF EARLY AND LATE LUNG SEQUELAE AFTER CONFORMAL RADIOTHERAPY IN BREAST CANCER PATIENTS

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Purpose: To study the risks of early and late radiogenic lung damage in breast cancer patients after conformal radiotherapy.
Methods and Materials: Radiogenic lung sequelae were assessed prospectively in 119 patients by means of clinical signs, radiologic abnormalities, and the mean density change (MDC) of the irradiated lung on CT.
Results: Significant positive associations were detected between the development of lung abnormalities 3 months or 1 year after the radiotherapy and the age of the patient, the ipsilateral mean lung dose (MLD), the radiation dose to 25% of the ipsilateral lung ($D_{25\%}$) and the volume of the ipsilateral lung receiving 20 Gy ($V_{20\ Gy}$). The irradiation of the axillary and supraclavicular lymph nodes favored the development of pneumonitis but not that of fibrosis. No relation was found between the preradiotherapy plasma TGF-β level and the presence of radiogenic lung damage. At both time points, MDC was strongly related to age. Significant positive associations were demonstrated between the risks of pneumonitis or fibrosis and the age of the patient, MLD, $D_{25\%}$, and $V_{20\ Gy}$. A synergistic effect of MLD, $D_{25\%}$, and $V_{20\ Gy}$ with age in patients older than 59 years is suggested.
Conclusion: Our analyses indicate that the risks of early and late radiogenic lung sequelae are strongly related to the age of the patient, the volume of the irradiated lung, and the dose to it.

Breast cancer, Conformal radiotherapy, Lung density, Radiation pneumonitis, Radiogenic lung fibrosis, Smoking, TGF-β.

INTRODUCTION

As a standard form of treatment after breast-conserving surgery, adjuvant radiotherapy is administered in a significant proportion of the patients and contributes to the decreasing mortality rate among the affected population (1, 2). Accordingly, it is vital that the frequency, extent, and severity of the side effects should be kept as low as possible.

The reported incidence of radiation-induced lung injury in breast cancer in prospective studies varies between 4.5% and 63% (3–8) and in retrospective studies between 0.9% and 30% (9–11). Early radiation-induced symptoms arise within 6 months after the completion of radiotherapy and may later progress to a chronic fibrotic status (12, 13). In older age, the irradiation of a larger lung volume and a higher mean lung dose (MLD) have been found to be risk factors for the occurrence of radiogenic lung damage (11, 14). The risk may be reduced through the use of conformal radiotherapy (15, 16), and under such conditions, the low rate of lung complications may be more dependent on patient-related variables (6).

Transforming growth factor (TGF)-β has been implicated as a key cytokine in the development of the radiation-related injury of various normal tissues, including the lung (17–24). Although the autocrine and paracrine actions of TGF-β are well established, the endocrine effects are vague. The expression of TGF-β may be induced by radiation (17, 18). Among other factors, its circulating level may reflect individual radiation sensitivity (18, 21, 23, 24).

We set out to perform a prospective analysis of the risks of early and late radiogenic lung damage in early breast cancer patients after conformal radiotherapy. The primary objective was to study radiogenic lung damage in relation to the various patient- and treatment-associated characteristics. The secondary objective was to investigate whether the plasma TGF-β level before or after the radiotherapy might

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serve to predict early or late radiogenic lung damage. We report here the results of this clinical study of 119 patients.

METHODS AND MATERIALS

The study had been approved by the Institutional Review Board of the University of Szeged, and all enrolled patients gave their written informed consent before being registered as participating in the study.

Study population

Between November 2001 and August 2004, 119 patients after curative surgery for breast cancer who required radiotherapy were recruited at our department. Systemic treatment was administered in accordance with the institutional guidelines. Perioperative chemotherapy was completed 4 weeks or more before the radiotherapy, and adjuvant hormonal therapy (tamoxifen or anastrozole), if necessary, was given simultaneously with radiotherapy. Patients with prior malignancy, pulmonary or autoimmune disease, any other significant health problem, or who were on glucocorticoid therapy were excluded. Data on smoking habits were collected, with the participants categorized as past or present smokers or nonsmokers.

Radiotherapy

CT-based three-dimensional treatment planning was performed for each patient. All patients were irradiated in the supine position, with both arms elevated above the head. The target volume and organs at risk (OARs) were contoured on the CT slices in a Nucletron TMS radiotherapy planning system (Nucletron B.V., Veenendaal, The Netherlands) according to the local protocol, based on the literature (5, 8, 16). Local (operated breast or chest wall) or locoregional (the former together with the coverage of any of the following regions: axillary, supraclavicular, and internal mammary lymph nodes [IMNs]) radiotherapy was chosen in accordance with the local protocol. For local irradiation we used two tangential photon beams, for the treatment of the IMNs a deep oblique field, and for the coverage of axillary and supraclavicular lymph nodes an anterior photon beam. Conformal radiotherapy was delivered by multiple 6/15-MV photon fields to the remaining breast parenchyma/chest wall and to the lymph nodes, if indicated, at a dose of $25 \times 1.8–2$ Gy; the tumor bed was irradiated with either 6-MV photon or 8–15-MeV electron fields when necessary. Prescribed OAR dose constraints were based on literature data (9, 16, 25–27) as follows: central lung distance (CLD) $\leq$3 cm, and MLD $<$20 Gy. We defined the percentage volume of the ipsilateral lung receiving at least 20 Gy ($V_{20\ Gy}$) as $<$25%, and endeavored to keep the volume of the ipsilateral lung irradiated with 25 Gy or more, under 25% ($D_{25\%} = 25$ Gy) as optimal thresholds. A maximum heart distance of 2 cm and a maximum heart volume receiving at least 25 Gy of $<$10% were also attempted.

The radiation dose to the contralateral breast was recorded. Radiotherapy was delivered with a linear accelerator (Mevatron KDS-2; Siemens AG, Berlin, Germany) in five fractions per week.

TGF-β determinations

Blood samples were collected from each patient as follows: on the day of the commencement of the radiotherapy, on the last day of irradiation of the breast/chest wall or lymph node regions, and 3 months later. Whole blood (6 mL) was drawn (without placing a tourniquet on the patient's arm) into tubes containing 7.5% K_3EDTA and immediately transferred on ice to the laboratory.

Plasma TGF-β levels were measured with a Quantikine ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The limiting sensitivity of the test was 7 pg/mL.

Evaluation of radiogenic lung damage

The patients were regularly seen at 3-month follow-up visits. At 3 months and 1 year after the completion of radiotherapy, CT examinations were performed. The CT scans were compared with those provided for radiotherapy planning purposes. Inflammatory or fibrotic abnormalities were diagnosed according to the accepted criteria, that is, increased density, hazy opacity, strandlike densities, or an onset of shrinkage for pneumonitis; thickened interlobular septae, subpleural strands, and fibrous intraparenchymal strands for fibrosis.

Early and late radiogenic lung damage was evaluated according to the Common Toxicity Criteria version 2.0. (The categories "pneumonitis of Grade 1" and "lung fibrosis of Grade 1" were used when radiographic changes occurred with or without pulmonary symptoms not requiring steroids; none of the patients in this study developed Grade 2 pneumonitis or fibrosis requiring steroids or diuretics.)

Lung density measurements were performed on each set of CT scans of a series of 94 patients, using an improved method based on that of Wennberg *et al.* (5). In 19 cases, at least one of the sets of CT scans was not available in a digital format appropriate for density measurements, and in 6 cases, CT was not performed 1 year after the radiotherapy because of disease progression ($n = 4$), sudden death ($n = 1$), or lack of patient compliance ($n = 1$). In the central slice (at the level of the left heart ventricle), lines were drawn between the edge of the sternum and the midheight of the ipsilateral chest wall on both sides, and mean lung density was measured in the areas defined by the lines and the chest wall (Fig. 1). In 48 patients who received locoregional irradiation, mean lung density was additionally measured in the entire area of both lungs in the apical slice at the level of the superior aspect of the head of the clavicle (Fig. 1). Rarely found inflammatory or fibrotic lesions on the postradiotherapy CT scans were excluded from the region of interest. To correct for lung density differences due to breathing, the contralateral mean lung density value was subtracted from that of the ipsilateral lung. The differences between the corrected ipsilateral lung densities at 3 months or 1 year after and before the radiotherapy were taken as the mean lung density changes at the level of the left heart ventricle (MDC_{LHV}) and the head of the clavicle (MDC_{HC}).

Statistical analysis

The various patient- and radiotherapy-related characteristics were examined according to the presence or the absence of Grade 1 pneumonitis or fibrosis or the change in lung density after the radiotherapy by univariate statistical methods: for the continuous variables Student's t test, and for the categorical variables the Chi-square test, followed by Fisher's exact test were utilized. The changes in MDC_{LHV} and MDC_{HC} or the TGF β levels were tested by repeated-measures analysis of variance. Spearman's coefficient of correlation was computed to examine the relationship between the variables. Multiple linear regression was used to examine the joint effect of the potential risk factors on lung density. Logistic regression models were applied to examine the potential risk

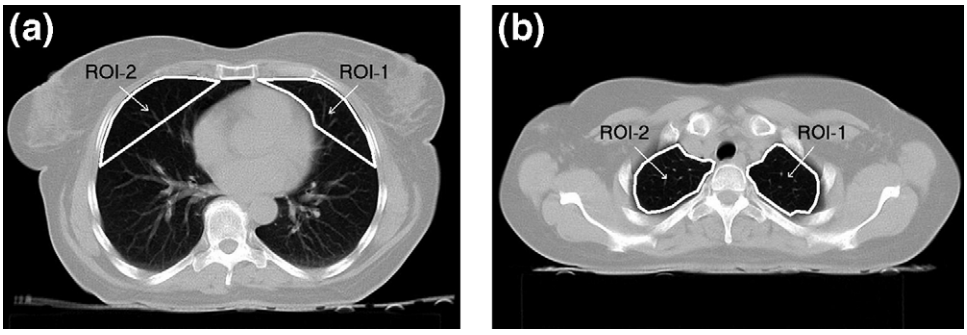

Fig. 1. Evaluation of lung density in the computed tomography slices at the levels of the left heart ventricle (a) and the superior aspect of the head of the clavicle (b). Mean lung density was measured in the region of interest (ROI) as outlined on both sides, and for the accurate assessment of lung density changes, the ipsilateral lung density was corrected by that on the contralateral side.

factors for the occurrence of Grade 1 pneumonitis or fibrosis: first, binary univariate logistic regression models used separately, followed by multivariate logistic regression models to examine the joint effects and the interactions. A stepwise procedure was used with a likelihood ratio test. Statistical analysis was performed with SPSS 11.0 for Windows and Statistica 6.1 for Windows (SPSS Inc., Chicago, IL).

RESULTS

The data on 119 patients were analyzed. The mean (± SE) age of the study population was 58.1 ± 1.0 (28.2–80.4) years; 34% of the patients had undergone mastectomy, and 66% tumor excision with sentinel lymph node biopsy and/or axillary lymph node dissection. The vast majority (96%) of the tumors were invasive, and two-thirds were invasive ductal cancers. Sixty-four (54%) were node-negative. Forty-two patients (35%) were past or present smokers. The distribution of the irradiated volumes and the resulting data concerning the dose to the lung are presented in Table 1.

Forty-four patients (37%) were categorized as having Grade 1 pneumonitis, but only 9 (7.5%) of them exhibited clinical symptoms. The occurrence of early lung damage was compared with the various patient- and radiotherapy-related characteristics (Table 2). Significant associations were found between the development of pneumonitis and the age of the patient ($p = 0.012$), MLD ($p = 0.003$), $V_{20\ Gy}$ ($p = 0.005$), and $D_{25\%}$ ($p < 0.001$). No difference in the CLD measures was found between the patients with or without early radiogenic lung changes. Radiotherapy of the supraclavicular and axillary lymph nodes favored Grade 1 pneumonitis ($p = 0.022$), but no such effect was found when the analysis was performed according to the inclusion or exclusion of the IMNs in the PTV. No significant difference was found in the occurrence of Grade 1 pneumonitis according to the type of the surgery. A past or present history of smoking was associated with a lower rate of early radiogenic lung damage ($p = 0.028$) (Table 2). Among the nonsmoker patients, 10% developed symptomatic pneumonitis and 35% exhibited radiologic changes; the corresponding rates in the group of past or present smokers were 2% and 19%, respectively.

Lung density measurements were also available at 3 and 12 months after radiotherapy in 94 cases at the level of the left heart ventricle (MDC_{LHV}) and in 48 cases with locoregional irradiation at the level of the head of the clavicle (MDC_{HC}), as well. The MDC_{LHV} values were increased significantly 3 months after the radiotherapy in patients with pneumonitis as compared with those without pneumonitis. No such difference was found for MDC_{HC} (Table 2).

Late radiation lung sequelae could be studied in 113 patients; 40 (35.4%) developed apparent minor fibrotic changes corresponding to Grade 1 fibrosis on CT scan, acquired 1 year after the completion of the radiotherapy, but none had clinical symptoms or needed medical treatment.

Table 1. Parameters reflecting the radiation dose to the ipsilateral lung after local or locoregional radiotherapy

Target volume	MLD (Gy)	$V_{20\ Gy}$* (%)	$D_{25\%}$† (Gy)
Breast ($n = 52$)	8.8 ± 0.4	16.2 ± 0.8	7.2 ± 1.1
Chest wall ($n = 3$)	8.7 ± 1.5	15.5 ± 3.2	5.3 ± 0.8
Breast/chest wall + supraclavicular + axillary lymph nodes ($n = 3$)	17.1 ± 1.5	33.6 ± 4.1	35.6 ± 6.1
Breast/chest wall + supraclavicular + axillary + internal mammary lymph nodes ($n = 61$)	17.1 ± 0.4	36.7 ± 1	34.1 ± 1

Abbreviation: MLD = mean lung dose.
* Volume of the ipsilateral lung receiving 20 Gy.
† Dose to 25% of the volume of the ipsilateral lung.

Table 2. Patient-related and therapy-related features among patients with or without Grade 1 pneumonitis

	No change	Grade 1 pneumonitis	*p*
Age (years)	56.2 ± 1.3	61.5 ± 1.5	0.012*
Type of surgery			
Excision	51 (66%)	26 (34%)	0.427†
Mastectomy	24 (57%)	18 (43%)	
Smoking			
Nonsmoker	42 (55%)	35 (45%)	0.010†
Past or present smoker	33 (79%)	9 (21%)	
RT of the supraclavicular and axillary nodes			
Yes	34 (53%)	30 (47%)	0.022†
No	41 (75%)	14 (25%)	
RT of the internal mammary nodes			
Yes	34 (56%)	27 (44%)	0.128†
No	41 (71%)	17 (29%)	
MLD (Gy)	12.2 ± 0.6	15.0 ± 0.7	0.003*
$V_{20\ Gy}$ (%)	24.8 ± 1.5	31.1 ± 1.6	0.005*
$D_{25\%}$ (Gy)	18.1 ± 1.8	27.7 ± 2.2	0.001*
CLD (cm)	2.7 ± 0.1	2.9 ± 0.1	0.202*
MDC_{LHV} (HU) at 3 months	67.0 ± 8.3	122.4 ± 14.3	0.001*
MDC_{HC} (HU) at 3 months	47.7 ± 10.9	65.3 ± 13.0	0.369*

Abbreviations: RT = radiotherapy; MLD = mean lung dose; V_{20Gy} = Volume of the ipsilateral lung receiving 20 Gy; $D_{25\%}$ = The dose at least delivered to 25% of the volume of the ipsilateral lung; CLD = central lung distance; MDC_{LHV} = mean density change of the irradiated lung at the level of the left heart ventricle; MDC_{HC} = mean density change of the irradiated lung at the level of the head of the clavicle.

* Student's *t*-test.

† Fisher's exact test.

The presence of inflammatory changes at 3 months was strongly correlated with that of fibrotic abnormalities at 1 year ($r = 0.733$, $p < 0.0001$). The inflammatory CT lesions detected at 3 months after the radiotherapy had disappeared 9 months later in 6 patients, and 3 patients developed new fibrotic lesions 1 year after the radiotherapy without having had early lung changes before that. The associations between the radiogenic pulmonary changes 1 year after the irradiation and the data relating to the patient or the radiotherapy are included in Table 3. Significant associations were found between the development of fibrosis and the age of the patient ($p = 0.006$), MLD ($p = 0.017$), $V_{20\ Gy}$ ($p = 0.034$), and $D_{25\%}$ ($p = 0.004$). We examined the risk of fibrosis depending on lung density changes with logistic regression. Significant associations were found between the development of fibrotic abnormalities and both MDC_{LHV} (odds ratio [OR] = 1.019, for every 1.0 unit increase; 95% CI, 1.01–1.028; $p < 0.0001$) and MDC_{HC} (OR = 1.013, for every 1.0 unit increase; 95% CI, 1.001–1.026; $p = 0.041$) measured 3 months after the radiotherapy. Significant associations were not found between the development of fibrotic changes and CLD, irradiation of the regional lymph nodes, the type of surgery, or smoking habit. The MDC_{LHV} values were increased significantly 1 year after the radiotherapy in those patients who displayed fibrotic lung changes. No such difference was found for MDC_{HC} (Table 3). MDC_{LHV} and MDC_{HC} were significantly reduced at 1 year compared with 3 months after the radiotherapy ($p < 0.001$).

Radiogenic lung damage was quantitatively analyzed via the lung density changes in relation to the patient- and radiotherapy-related parameters. MDC_{LHV} at 3 months was correlated with age ($r = 0.2$, $p = 0.046$) and MDC_{LHV} at 1 year ($r = 0.564$, $p < 0.001$), but not with MLD, $V_{20\ Gy}$, $D_{25\%}$, or CLD. MDC_{LHV} at 1 year, however, in addition to age ($r = 0.278$, $p = 0.007$), was significantly correlated with MLD ($r = 0.206$, $p = 0.047$), $V_{20\ Gy}$ ($r = 0.214$, $p = 0.039$), and $D_{25\%}$ ($r = 0.214$, $p = 0.039$) but not with the other parameters. The MDC_{HC} values at 3 months and 1 year correlated significantly ($r = 0.521$, $p < 0.001$) but were not related to any of the other parameters.

The initial plasma TGF-β levels and those at the termination of radiotherapy were not related to radiogenic lung damage at 3 months or 1 year after the radiotherapy. We found correlations between the TGF-β level and MDC_{LHV} ($r = 0.264$, $p = 0.029$) and MDC_{HC} ($r = 0.622$, $p = 0.002$) 3 months after the radiotherapy. Those patients who developed symptomatic pneumonitis had significantly higher TGF-β levels 3 months after the radiotherapy than the patients with no CT changes ($p = 0.006$) or the patients who developed asymptomatic CT abnormalities ($p = 0.028$) (Fig. 2). The plasma TGF-β level was not associated with smoking.

To estimate the risk of pneumonitis or fibrosis, the effects

Table 3. Patient-related and therapy-related features among patients with or without Grade 1 fibrosis

	No fibrosis	Fibrosis	*p*
Age (years)	56.7 ± 1.2	62.4 ± 1.5	0.006*
Type of surgery			
Excision	46 (63%)	27 (37%)	0.685†
Mastectomy	27 (68%)	13 (32%)	
Smoking			
Nonsmoker	43 (59%)	30 (41%)	0.103†
Past or present smoker	30 (75%)	10 (25%)	
RT of the supraclavicular and axillary nodes			
Yes	33 (57%)	25 (43%)	0.115†
No	40 (73%)	15 (27%)	
RT of the internal mammary nodes			
Yes	33 (60%)	22 (40%)	0.333†
No	40 (69%)	18 (31%)	
MLD (Gy)	12.2 ± 0.6	14.5 ± 0.7	0.017*
$V_{20\ Gy}$ (%)	24.7 ± 1.4	29.8 ± 1.8	0.034*
$D_{25\%}$ (Gy)	17.9 ± 1.7	26.2 ± 2.4	0.004*
CLD (cm)	2.7 ± 0.1	2.8 ± 0.1	0.412*
MDC_{LHV} (HU) at 1 year	31.5 ± 9.76	95.8 ± 17.91	0.0001*
MDC_{HC} (HU) at 1 year	25.8 ± 6.0	35.2 ± 9.9	0.38*

Abbreviations as in Table 2.

* Student's *t*-test.

† Fisher's exact test.

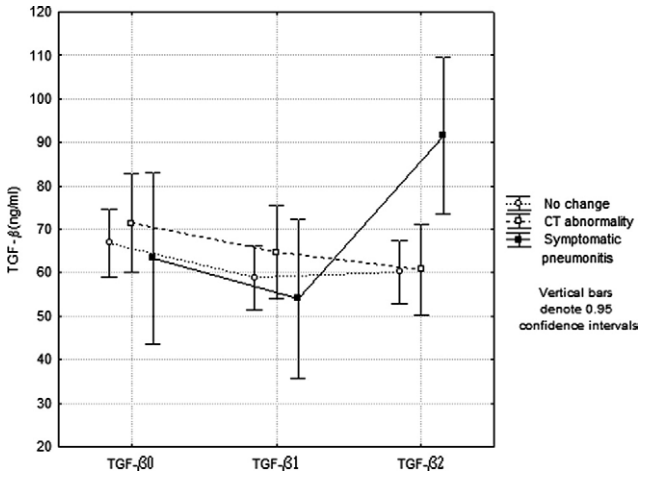

Fig. 2. Circulating TGF-β level in patients with or without radiation lung damage before (TGF-β0), at the end of (TGF-β1) and 3 months after the radiotherapy (TGF-β2).

of the age of the patient, MLD, $V_{20\ Gy}$, $D_{25\%}$, and irradiation of the supraclavicular and axillary lymph nodes were first studied in separate univariate logistic regression models, resulting in crude odds ratios (Tables 4 and 5). The risk of Grade 1 pneumonitis or fibrosis increased with the age of the patient. Significant positive associations were demonstrated between the risks of pneumonitis or fibrosis and MLD, $D_{25\%}$, and $V_{20\ Gy}$. Irradiation of the axillary and supraclavicular lymph nodes increased the risk of pneumonitis but did not significantly influence the risk of Grade 1 fibrosis (Tables 4 and 5). Multivariate logistic regression analysis was applied, including one of the dose parameters (MLD, $D_{25\%}$ and $V_{20\ Gy}$) or the irradiation of the axillary and supraclavicular lymph nodes and age in separate models to examine their effects and interactions using a stepwise algorithm. This strategy was followed because of the strong correlation between the variables related to irradiation of the lung. The effects of the age, the irradiation of the axillary and supraclavicular lymph nodes, and the parameters indicating the radiation dose to the lung remained significant in each case, but their interaction with age was not significant. Thus, all the dose parameters and the irradiation of the axillary and supraclavicular lymph nodes, when adjusted for age, predicted the occurrence of pneumonitis and fibrosis but did not show synergism in the overall population. The adjusted odds ratios are shown in Tables 4 and 5.

We followed the approach of Gagliardi *et al.* (11) in testing whether use of the median age of the study population as a threshold would better identify patients at high risk of early lung complications. Patients older than 59.33 years had OR = 2.48 (95% confidence interval [CI], 1.15–5.35; $p = 0.018$) and OR = 3.34 (95% CI, 1.47–7.60; $p = 0.004$) for a higher risk of radiation pneumonitis or fibrosis, respectively. When this age limit was combined with MLD or the irradiation of the axillary and supraclavicular lymph nodes, significant interactions were found for both pneumonitis (OR = 1.27, for every 1.0-Gy increase, 95% CI, 1.11–1.46; $p < 0.0001$ for MLD, and OR = 7.65, 95% CI, 2.98–19.62; $p < 0.0001$ for inclusion of the lymph nodes into the irradiated volume, respectively) and Grade 1 fibrosis (OR = 1.12, for every 1.0-Gy increase, 95% CI, 1.06–1.19; $p < 0.0001$ for MLD and OR = 6.43; 95% CI, 2.53–16.37; $p < 0.0001$ for inclusion of the lymph nodes into the irradiated volume, respectively). When MLD was replaced in the model by either $V_{20\ Gy}$ or $D_{25\%}$, the results were very similar (data not shown).

DISCUSSION

We found a 37% incidence of radiation Grade 1 pneumonitis and a 35.5% incidence of Grade 1 radiation fibrosis in breast cancer patients after conformal adjuvant radiotherapy. Only nine of the patients with early lung reactions exhibited clinical symptoms, and no patient had respiratory problems due to lung fibrosis. The strongest risk predictor for radiation lung sequelae was the age of the patient. The parameters reflecting the dose to and the volume of the irradiated lung were associated with the risk of pulmonary complications and displayed synergism with the age in patients >59 years old. In patients with pneumonitis, ele-

Table 4. OR and 95% CI for pneumonitis with regard to the age of the patient and the radiotherapy-related parameters

	Crude			Adjusted for age		
Variable	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.05	1.01–1.09	0.015			
MLD	1.13	1.04–1.22	0.004	1.16	1.07–1.27	0.001
$V_{20\ Gy}$*	1.04	1.01–1.08	0.008	1.06	1.02–1.10	0.002
$D_{25\%}$†	1.04	1.02–1.07	0.002	1.05	1.02–1.08	<0.0001
$RT_{supra\text{-}axilla}$‡	2.59	1.18–5.64	0.015	3.13	1.38–7.10	0.006

Abbreviations: CI = confidence interval; MLD = mean lung dose; OR = odds ratio.
For continuous variables, OR indicates the risk for every one–unit increase in that variable.
* Volume of the ipsilateral lung receiving 20 Gy.
† Dose to 25% of the volume of the ipsilateral lung.
‡ Irradiation of the supraclavicular and axillary lymph nodes.

Table 5. OR and 95% CI for fibrosis with regard to age and the radiotherapy-related parameters

	Crude			Adjusted for age		
Variable	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.06	1.02–1.10	0.008			
MLD	1.10	1.02–1.10	0.019	1.13	1.04–1.24	0.006
$V_{20\ Gy}$*	1.04	1.01–1.06	0.008	1.04	1.02–1.07	0.002
$D_{25\%}$†	1.04	1.02–1.07	0.036	1.05	1.01–1.08	0.011
$RT_{supra-axilla}$‡	2.02	0.92–4.46	0.081	2.32	1.01–5.28	0.046

Abbreviations: CI = confidence interval; MLD = mean lung dose; OR = odds ratio.
For continuous variables, OR indicates the risk for every one–unit increase in that variable.
* Volume of the ipsilateral lung receiving 20 Gy.
† Dose to 25% of the volume of the ipsilateral lung.
‡ Irradiation of the supraclavicular and axillary lymph nodes.

vated circulating levels of TGF-β were found 3 months after the radiotherapy.

Radiation-induced lung injury, both early as radiation pneumonitis and late as radiation fibrosis, has been studied extensively. The incidence of radiation pneumonitis varies between 0.9 and 80% (3, 4, 7–10, 28–30) and is obviously lessened by the performance of conformal radiotherapy (4, 5, 8, 15, 16). The frequencies of pneumonitis after conformal radiotherapy in other series were 0.9–11% (3), 8% (8), 10–26% (6), 23% (5), 29% (11), and 47% (4). Radiogenic fibrosis was diagnosed in 6.4% (8), 22.1% (30), and 87% (7) of patients in other studies. One reason for such a wide range of incidence of pulmonary complications is the various grading systems and endpoints used. Whereas some investigators defined the endpoint of their study as symptomatic pneumonitis and counted only those cases with Grade 1 pneumonitis that produced pulmonary symptoms (5, 6, 11, 28), others reported on the radiologic changes without regard to the symptoms (7, 30). Another factor that may influence the findings is the prospective nature of a study favoring the more frequent diagnosis of radiogenic lung damage (3–8) compared with that in retrospective trials (9–11, 28, 30). Whereas about one third of the patients in our prospective study had developed radiographic changes by 3 months and 1 year after the radiotherapy, only a minority suffered from symptoms that were easily managed, and none had respiratory problems 1 year after the radiotherapy. Thus, in accord with the literature, we found that significant radiogenic lung sequelae after conformal radiotherapy in breast cancer were rare.

We computed the risk of radiogenic lung damage in relation to the age of the patient and the various data reflecting the dose to the lung and found significant, although modest, increases connected with these parameters. Age exerted the strongest effect on the risk of radiation lung complications. This finding is consistent with the results of other studies on breast cancer patients (5, 11, 14, 30). Using the relative seriality model, Gagliardi *et al.* demonstrated that with the median age of their study population as a limit, the dose to the lung that gives a complication probability (Normal Tissue Complication Probability) of 50% is 40.6 Gy for patients <57 years and 26.9 Gy for patients >57 years old (11). We tested whether use of the median age of the study population as a threshold would better identify patients at high risk for lung complications. Patients older than the median (59.3 ± 1.0 years) had risk of radiation pneumonitis and fibrosis that were approximately 2.5 and 3 times higher, respectively. Moreover, above this age limit, we demonstrated synergism between MLD, $V_{20\ Gy}$, $D_{25\%}$, and the age of the patient, indicating the need for heightened attention when radiotherapy is to be delivered to patients aged over age 59. The elaboration of age-adjusted dose constraints on the basis of a large enough data set, developed by each institute according to its patient population, methods, and experience, is proposed.

The probability of lung complications may be improved through the use of conformal radiotherapy (15, 16). A widely studied predictor of the risk of radiogenic lung damage is MLD, which was found to be the most accurate predictor of radiation pneumonitis in the study by Seppenwoolde *et al.* (25). In an analysis of 59 breast cancer patients, the incidence of radiation pneumonitis greater than Grade 1 was around 10% at an MLD level of 20 Gy (9). Guidelines defining strict dose constraints for the conformal radiotherapy of breast cancer are lacking. We used MLD ≤20 Gy as a dose constraint for the ipsilateral lung and studied the roles of $V_{20\ Gy}$ and $D_{25\%}$ in the prediction of radiogenic lung sequelae. Strong correlations were found between these parameters, and all were related to the risk of radiation lung damage.

Another parameter obviously related to radiogenic lung damage is CLD (9, 10, 28). We kept CLD strictly <3 cm, which is probably why we did not find a significant effect of CLD on the risk of radiogenic lung complications. If 3D conformal radiotherapy is applied, analysis of the parameters reflecting the dose to or the volume of the irradiated lung may predict radiation pneumonitis better than CLD, although the latter is an excellent safety marker.

Inclusion of the lymph nodes in the irradiated volume clearly increases the radiation dose to the lung. Accordingly, irradiation of the regional lymph nodes has been demonstrated in numerous studies to increase the risk of

radiation pneumonitis (3, 5, 7, 10, 28). Ooi *et al.* (7) observed a very high incidence of radiation pneumonitis, deterioration of the lung function indices, and the occurrence of chest X-ray opacities following the delivery of locoregional radiotherapy in 30 breast cancer patients. In a study of 121 breast cancer patients, symptomatic radiation pneumonitis developed exclusively in those who received locoregional irradiation (5). We found that irradiation of the axillary and the supraclavicular lymph node regions is associated with a 2.5-times higher risk of radiation pneumonitis and a twofold risk of radiogenic fibrosis.

We used two methods for the evaluation of radiogenic lung changes on the CT scans. The quantitative assessment of lung density changes provides information different from that yielded by inspection of the CT scans. Whereas the former reflects the overall effect of radiation on the lung, the latter reveals localized inflammatory or fibrotic changes that may be resulted by the overdosage in small lung volumes. We modified the method of Wennberg *et al.* (5) for lung density measurements because we believe our method provides more accurate data on the density changes of the irradiated lung than the original for two reasons: first, lung density variability related to breathing is corrected when the density of the contralateral lung is included in the calculations; second, the performance of density assessments specifically in the irradiated lung region, which is especially small when conformal radiotherapy is delivered, makes the method more sensitive and accurate than if the ventral third of the lung volume is used for measurements, as described in the original method. We suggest this improved method for the quantitative analysis of small differences in lung density after the adjuvant radiotherapy of breast cancer.

We found the lung density to be significantly lower 1 year after radiotherapy than after 3 months. Skoczylas *et al.* (12) noted that, following radiotherapy, lung density underwent an initial increase, then decreased, and then reached a stable level by 1 year in the majority of their early breast cancer patients. Nonetheless, in a few patients, the lung changes progressed over years, without an initial early phase. In fact, we also had three patients, without prior inflammatory abnormalities, who developed *de novo* fibrotic changes 1 year after radiotherapy. Our observation is in agreement with the theory that early and late radiogenic lung damage may develop independently (12, 13). It is a widely accepted practice to evaluate radiation fibrosis at 12 months (as a minimum time interval) after the irradiation (7, 12, 30). However, because the lung changes may progress for even 5–6 years, a longer follow-up is sometimes needed to establish the ultimate level of late lung damage (12).

We observed that the irradiation of the axillary and supraclavicular lymph nodes increased the risk of pneumonitis and fibrosis. Nonetheless, the MDC_{HC} values reflecting the inflammatory or fibrotic reaction of the irradiated apical lung in those patients who received locoregional radiotherapy did not appear to be related to any clinical or dosimetric parameters. Some clinical studies have demonstrated that because of spatial differences in lung radiosensitivity, the caudal lung regions are at higher risk of radiation pneumonitis than the cranial parts (14, 29). Although caution is called for because of the smaller number of patients with supraclavicular irradiation, these results lead us to speculate that the independence of the MDC_{HC} of the usual risk factors in our study is an indicator of the reduced radiosensitivity of the apical lung parenchyma, compared with that of other regions of the lung.

From an evaluation of the various NTCP models in a large group of breast cancer patients, Tsougos *et al.* (6) concluded that because the incidence of lung complications is low, the variation of interpatient radiosensitivity plays a significant role. Various efforts have been made to find particular radiosensitivity indices (19–23, 29–33). TGF-β, a multifunctional cytokine implicated in both tumor progression and normal tissue damage, has been studied as a potential predictive marker of radiogenic sequelae in breast cancer (19, 23) and lung cancer patients (21, 22, 31–33). TGF-β is abundant, and its receptor is ubiquitously expressed in all tissues including various malignant tumors (18, 20). Among other biologic activities, TGF-β, regulates differentiation of fibroblasts, extracellular matrix production, and angiogenesis (17, 18, 20). The expression of TGF-β in normal tissues may be induced by chemotherapy or radiotherapy (17, 18). The development of breast fibrosis was found to be significantly related to the preradiotherapy plasma TGF-β level in breast cancer (23). Measurement of the plasma TGF-β levels after the delivery of 73.6 Gy to lung cancer patients allowed the selection of those who could be treated beyond the conventional dose without the risk of radiation complications (21, 22). Novakova-Jiresova *et al.* (31) detected a trend to increased TGF-β levels in lung cancer patients who developed radiation pneumonitis by the third week of radiotherapy. The increase of TGF-β was correlated with MLD (32) and V30 (33) in lung cancer patients. We did not find TGF-β to be a predictor of the risk of early or late radiogenic lung changes. In fact, in patients with symptomatic pneumonitis, the TGF-β level was elevated 3 months after the radiotherapy, which should be regarded as a consequence of the pneumonitis. The inconsistency between our finding and that of others—that is, the absence of fluctuation in the plasma TGF-β level, might be explained by the relatively small irradiated lung volumes in our patients. This explanation is supported by the recent findings of Evans *et al.* (33) showing that TGF-β concentration is generally not predictive for radiation pneumonitis except when large lung volumes are irradiated.

The existence of an association between smoking and radiation lung injury in breast cancer patients is controversial (5, 14, 34, 35). Johansson *et al.* (35) and Theuws *et al.* (14) found lower incidence of radiation pneumonitis in smoking breast cancer patients (14, 35), malignant lymphoma patients (14), and esophagus cancer patients (35) compared with nonsmokers. Similarly, postradiotherapy lung inflammatory reactions were less intense in smoker than in nonsmoker lung cancer patients (36). In contrast, Wennberg *et al.* did not find significant differences in the

incidence of pneumonitis or the change in lung density after radiotherapy according to the smoking habits of 118 breast cancer patients (5). We observed significantly fewer cases of pneumonitis among smokers than nonsmokers. Following selection of the patients with symptomatic pneumonitis, the difference was even larger. One explanation of our findings could be the immunosuppressive effects of cigarette smoking and nicotine (37). In accord with the published data (34), no effect of smoking on radiation fibrosis was detected. Our findings extend the data on the relation of smoking and radiogenic lung damage and provide new information by demonstrating the lack of a dependence of the plasma TGF-β level on the smoking habits.

In conclusion, our analyses indicate that the strongest risk factor for early radiogenic lung damage is the age of the patient. The volume of the irradiated lung and the dose to it are also significant risk predictors, which exert synergistic effects with age in patients older than 59 years. Hence, primarily these parameters should be censored when adjuvant radiotherapy is delivered to early breast cancer patients.

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